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Otariodibacter oris and *Bisgaardia* Genomospecies 1 Isolated from Infections in Pinnipeds

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ABSTRACT: We document the first associations of two recently described species of *Pasteurellaceae* bacteria with lesions in wild pinnipeds in rehabilitation. Samples were collected from nine lesions in four California sea lions (*Zalophus californianus*) and two Pacific harbor seals (*Phoca vitulina*) during necropsy or admission examinations at a rehabilitation facility in northern California. Seven *Pasteurellaceae* isolates were identified using phenotypic tests and partial *rpoB* gene sequencing. Six strains of *Otariodibacter oris* were isolated from California sea lions. *Otariodibacter oris* was isolated in pure culture from four abscesses, an affected lymph node, and a bone lesion consistent with osteomyelitis. *Otariodibacter oris* was also cultured with *Arcanobacterium phocae* and β-hemolytic streptococci. A pure culture of *Bisgaardia* genomospecies 1 was obtained from an abscess in a harbor seal. This is the first time, to our knowledge, that *O. oris* has been associated with infection. Isolation of these bacteria in pure culture from abscesses and osteomyelitis strongly indicates a pathogenic potential of this organism. Likewise, the isolation of *Bisgaardia* genomospecies 1 in pure culture from an abscess in a harbor seal implies causality.

Key words: *Bisgaardia*, California sea lion, harbor seal, infection, *Otariodibacter oris*, *Pasteurellaceae*, pinnipeds.

Bacterial infections commonly cause morbidity and mortality in pinnipeds as primary and secondary pathogens (Higgins, 2000; Dunn et al., 2001). Recently, a new genus consisting of one species, *Bisgaardia hudsonensis*, and one genomospecies, *Bisgaardia* genomospecies 1, was isolated from healthy and diseased seals (*Phocidae*; Foster et al., 2011; Hansen et al., 2012a). Bacteria belonging to a new genus consisting of one species, *Otariodibacter oris*, were isolated from the oral cavity of healthy sea lions (*Otariinae*), fur seals (*Arctocephalinae*), and walruses (*Odobenidae*; Hansen et al., 2012a; Hansen et al., 2012b).

Pasteurellaceae have been associated with bite-wound infections in animals and humans, often resulting in generalized infections (Holst et al., 1992; Smith et al., 2000). Given that both *O. oris* and *Bisgaardia* species are part of the normal oral microflora in pinnipeds, it is likely that these bacteria can cause infection through bites from these animals. *Bisgaardia hudsonensis* was recently isolated from an infected seal bite in a human (Sundeep and Cleeve, 2011). We investigated the role of *Pasteurellaceae* bacteria in the etiology of abscessation and disseminated infection in six pinnipeds.

We used BBL culture swabs (BD Biosciences, Le Point de Claix, France) to collect samples opportunistically during necropsy or admission examinations of four California sea lions and two Pacific harbor seals at The Marine Mammal Center (TMMC; Sausalito, California, USA) in June 2011. Stranded pinnipeds were evaluated as described by Greig et al. (2005) and examined clinically before sampling.

Case 1: A female, California sea lion yearling was found outside of typical sea lion habitat, with reduced use of the hind flippers, moderately underweight, and multiple subcutaneous abscesses. An abscess was sampled on arrival at TMMC.

Case 2: A female California sea lion was found emaciated and lethargic with an abscess on the right hind flipper. A sample was collected from the abscess.

Case 3: A female, California sea lion yearling was found lethargic and in renal failure and was euthanized because of poor prognosis. The major necropsy findings were multifocal, cutaneous and subcutaneous abscesses, 0.5–1 cm in diameter, on the abdomen and hind flippers, one of which was sampled. The kidneys were bilaterally swollen with gross changes typical of leptospirosis (Greig et al., 2005); the spleen was congested, and the liver was friable.

Case 4: A subadult, male California sea lion was found mildly underweight with an injury to the left front flipper. The animal died after arrival at TMMC, and at necropsy, 75% of the left front flipper was necrotic with exposure of muscle and bones. The chest had a 30-cm-diameter, subcutaneous abscess with abundant red/brown, malodorous, purulent fluid.

The sternum was fractured and had lesions consistent with osteomyelitis adjacent to the third and fourth ribs, with surrounding soft tissue necrosis. The third–seventh right ribs were fractured close to the sternal attachments and the right axillary lymph node was enlarged with central necrosis. The liver was friable, and the kidneys and lungs were enlarged and edematous. Swab samples were collected from the abscess on the chest, the left subscapular lymph node, and from the affected sternum.

Case 5: A female, harbor seal pup found obtunded on a beach was dead on arrival at TMMC. At necropsy, skeletal muscle of the left shoulder region contained a 30-cm-diameter abscess with central, brown/pale-green, purulent material, and the associated scapula-humeral joint was necrotic. In the right hind flipper, from the tarsus to the stifle, soft tissues and joint spaces contained pale-green, purulent fluid. The lungs were edematous with multifocal, necrotic foci, and the lateral and medial lobes of the liver had large regions of necrosis. Swab samples were collected from the left shoulder abscess and affected joints in the left hind flipper.

Case 6: A male, harbor seal pup was found lethargic and underweight with an umbilical abscess that was sampled.

Swabs were kept in Stuart liquid medium (BD Biosciences) at 5 C for up to 6 hr before plating on 5% bovine blood agar (Hardy Diagnostics, Santa Maria, California, USA). Blood agar plates were incubated aerobically in sealed, plastic bags for 24 hr at 37 C. Colonies typical of *Pasteurellaceae* were subcultured and characterized as described (Christensen et al., 2007).

The Easy-DNA kit (Invitrogen, Carlsbad, California, USA) was used for DNA isolation according to the manufacturer's instructions. The partial *rpoB* gene sequence was obtained from seven isolates (Table 1), as reported previously (Korczak et al., 2004). Sequencing was performed by Macrogen (Gasan-dong, Seoul, Korea). Resulting sequences were compared with existing gene sequences in GenBank using BLAST (Benson et al., 2007). Pairwise comparisons were performed in the program WATER included in EMBOSS (Rice et al., 2000).

Five isolates (Table 2) were tested for antimicrobial susceptibility using a microbroth dilution assay, Sensititre COMPAN1F (Trek Diagnostic Systems Inc., Westlake, Ohio, USA) as described by the manufacturer and in accordance with recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2008). The inocula were prepared in veterinary fastidious medium (VMF). *Histophilus somni* ATCC 700025 was included as control.

Results for the six cases are listed in Table 1. This is the first isolation of *O. oris* from lesions, to our knowledge. The finding of the bacteria in pure culture from abscesses and osteomyelitis indicates that *O. oris* can cause soft tissue and bone infections. Likewise, because *Bisgaardia* genomospecies 1 was isolated in pure culture from an abscess in a harbor seal, it is very probable that it was the cause of the lesion.

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TABLE 1. *Pasteurellaceae* strains isolated from infections in California sea lions (*Zalophus californianus*), and Pacific harbor seals (*Phoca vitulina*) at a rehabilitation facility in California, USA, June 2011.

Case no.	Strain	Strain ID	Animal ID	*Pasteurellaceae* taxon	*rpoB* similarity with type strain	Host	Site of isolation
1	Egwene2	KC594689	CSL 9959	*Otariodibacter oris*	98%	California sea lion	Subcutaneous abscess, mixed culture with *Arcanobacterium phocae* and β-hemolytic streptococci.
2	Mandarain1	KC594691	CSL 9970	*Otariodibacter oris*	98%	California sea lion	Abscess on right hind flipper, pure culture.
3	Sharla	KC594692	CSL 9962	*Otariodibacter oris*	97%	California sea lion	Dermal abscess, mixed culture with β-hemolytic Streptococcus.
4	Vimana1	Vimana2	CSL 9971	*Otariodibacter oris*	97%	California sea lion	Abscess chest, pure culture.
4	Vimana2	KC594690	CSL 9971	*Otariodibacter oris*	97%	California sea lion	Osteomyelitis in sternum, pure culture.
4	Vimama3	Vimana2	CSL 9971	*Otariodibacter oris*	97%	California sea lion	Lymph node subscapularis (left side), pure culture.
5	Macsue1	KC594693	HS 2177	*Bisgaardia* genomosp. 1	99%	Pacific harbor seal	Abscess in the left shoulder, pure culture.
5	Macsue4	None	HS 2177	Not *Pasteurellaceae*	—	Pacific harbor seal	Abscess in left hind flipper, β-hemolytic streptococci, pure culture.
6	Mike2	None	HS 2132	Not *Pasteurellaceae*	—	Pacific harbor seal	Abscess, *Arcanobacterium phocae*, pure culture.

The three isolates obtained from different lesions in one sea lion (case 4) showed 100% *rpoB* similarity within the group, but only 96% similarity with the *O. oris* strain isolated from the oral cavity of that animal, indicating that the bacteria in the lesions did not spread from the oral cavity flora. Likewise, the four other strains isolated from abscesses, only showed 96–97% similarity with the strain isolated from the oral cavity of the host animal. Because *O. oris* and *Bisgaardia* spp. are part of the normal microflora in the oral cavity of these animals (Hansen et al. 2012a), it is likely that the bacteria were transferred through a bite from an animal of the same species.

Given these findings, it is surprising that *Bisgaardia* spp. and *O. oris* are not reported more frequently from infections in pinnipeds. However, *Pasteurellaceae* are fastidious bacteria, and often require specific growth factors outside the host (Schwarz, 2008). Often primary plates in diagnostic laboratories are incubated aerobically or anaerobically, but not in a plastic bag or other conditions that provide a higher CO_2 concentration and humidity favoring growth of *Pasteurellaceae*. Therefore, it is likely that *Pasteurellaceae* often fail to grow during routine diagnostics. Also, our results indicate that *Bisgaardia* spp. and *O. oris* are often found in mixed cultures; thus, other bacterial growth may be detected that could be attributed as the causative agent.

In the literature describing infections in pinnipeds, methods for identifying pathogens are often not stated (Howard et al., 1983) or are based on phenotypic characters without genotypic confirmation (Thornton et al., 1998; Dunn et al., 2001). Also, Analytical Profile Index kits (API) that are used in many diagnostic laboratories have misidentified *Pasteurellaceae* (Collins et al., 1981), and the API databases contain few *Pasteurellaceae*, most of which are associated with human infections.

For the above reasons, it is likely that *O. oris* and *Bisgaardia* spp. are common

TABLE 2. Results of investigation of antimicrobial susceptibility of *Pasteurellaceae* isolated from California sea lions (*Zalophus californianus*) and Pacific harbor seals (*Phoca vitulina*) at a rehabilitation facility in California, USA, June 2011. The minimal inhibitory concentration (MIC) values are given for four *Otariodibacter oris* strains (Vimana2, Sharla, Egwene, and Mandarain1) and one *Bisgaardia* genomospecies 1 (Macsuel).[a]

Agent (Dilution range, µl/mL)	Dilution range (µl/mL)										
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64
Amikacin (4-32)							4/x				
Ampicillin (0.25-16)			x					1	3		
Cefazolin (4-16)							4/x				
Cefovecin (0.25-8)			4/x								
Cefoxitin (2-16)						4/x					
Cefpodoxime (2-16)						4/x					
Ceftiofur (0.25-4)			3/x	1							
Chloramphenicol (4-16)							4/x				
Clindamycin (0.5-4)				1/x	3						
Doxycycline (2-8)						3/x	1				
Enrofloxacin (0.25-2)			1	2/x	1						
Erythromycin (0.5-4)				4/x							
Gentamicin (1-8)					1/x	3					
Imipenem (1-8)					4/x						
Marbofloxacin (0.25-2)			4/x								
Oxacillin+ 2% NaCl (0.25-4)							4/x				
Penicillin (0.06-8)		2	1	1/x							
Rifampin (1-2)					4/x						
Ticarcillin (8-64)								1/x	3		
Amoxicillin/clavulanate acid 2:1 ratio (4/2-32/16)	3/x	1									
Ticarcillin/clavulanic acid (8/2-64/2)					3/x	1					
Trimethoprim/sulfamethoxazole (0.5/9.5-2/38)									3/x	1	

[a] The *Bisgaardia* genomospecies 1 strain values are marked with an x. The vertical lines indicate breakpoints. The vertical line to the left (or if there is only one line) represents the breakpoint between sensitive or intermediate resistance and the one to the right represents the breakpoint between intermediate resistance and resistant. The only exception is Oxacilin+2% NaCl, where the vertical line represents the breakpoint between sensitive and resistance. The MIC was defined as the lowest antibiotic concentration that resulted in no growth of the isolate. An isolate was considered susceptible to an antibiotic if the MIC of that antibiotic was less than the susceptibility breakpoint concentration described by the Clinical and Laboratory Standards Institute (CSLI) or the European Committee on Antimicrobial Susceptibility Testing (cefovecin, cefoxitin, and doxycycline) for each antimicrobial (CLSI, 2008; European Committee on Antimicrobial Susceptibility, 2011). Specific clinical breakpoints were used when available.

pathogens in pinnipeds and can cause localized and multicentric lesions. A more comprehensive study that includes a larger number of animals is warranted. Our findings also provide further support for the host specificity and possible coevolution of *Otariodibacter* and *Bisgaardia* among sea lions and seals, respectively.

Results for antimicrobial susceptibility testing are listed in Table 2. Although only a few strains were tested for antimicrobial susceptibility, *Otariodibacter oris* were resistant to ampicillin and oxacillin+2% NaCl, and intermediately sensitive to clindamycin, doxycycline, enrofloxacin, penicillin, and trimethoprim/sulfamethoxazole; all of which are commonly used therapeutics in the clinic. The *Bisgaardia* genomospecies 1 was resistant to oxacillin+2% NaCl and showed an intermediate reaction to penicillin, which, again, is important because these compounds are often used for treatment of infections. To our knowledge, this is the first report on

the antimicrobial susceptibility of *O. oris* and *Bisgaardia* genomospecies 1.

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